PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No.:	10/509,247
Filing Date:	September 28, 2004
Applicant:	Shunichi KURODA et al.
Group Art Unit:	1648
Examiner:	Peng, Bo
Title:	DRUGS COMPRISING PROTEIN FORMING HOLLOW NANOPARTICLES AND THERAPEUTIC SUBSTANCE TO BE TRANSFERRED INTO CELLS FUSED THEREWITH
Attorney Docket:	12480-000068/US

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

1. I, Shunichi Kuroda, a resident at the address of 7-C-104 Kamiyamada Suita-Shi Osaka, declare as follows:

2. I am a Doctor of Agriculture, and have experience in this field since 1984.

3. I am presently employed at Osaka University Industrial Science Institute as an Associate Professor, and have been working in the current department since 1998.

4. I have been granted seven (7) Patents in this field (6 Korean Patents and 1 Japanese Patent) and have written about 90 papers in this field (e.g. Nature Biotechnology).

5. Because of my own experience evidenced above, I believe myself to be one of at least ordinary skill in the art of nature biotechnology. Based upon the following experimental data which I conducted, it is my opinion that one of ordinary skill in the art would be convinced that the utility of the drug in which IFN or HGF is fused to HBsAg protein is sufficiently established.

6. **Data of Experiment regarding physiological activity of a drug in which IFN or HGF is fused to HBsAg protein**

6a. Physiological activity of HBsAg L protein fused with human interferon ω

A hepatic tissue (about $10mm^2$) derived from a patient of human hepatic cancer producing hepatitis C virus surface-antigen protein was subcutaneously injected into the renis of a nude mouse, and constant production of HCV-RNA qualitative method (Nippon Roche (NRRC)). Next, purified human interferon ω (IFN ω) expressed by E. coli bacteria was injected subcutaneously into the back of a mouse in an amount of blood concentration of about 100ng/ml using an osmotic pump. Then, the concentration of HCV-RNA in the blood was measured as needed. After a week, HCV-RNA was not detected from the blood.

Further, in the case of dosing IFN ω in an amount of blood concentration of about 10ng/ml, the disappearance of HCV-RNA from the blood after a week was not seen.

In contrast, in the case of dosing purified HBsAg L protein fused with IFN ω into the mouse in an amount of blood concentration of about 1ng/ml, the disappearance of HCV-RNA from the blood appeared immediately after a week.

Accordingly, by fusing IFN ω with HBsAg L protein, the dose of IFN ω with respect to HCV was reduced to 1/100 or less at least in a living organism. More specifically, it was confirmed that when the target-cell substance IFN ω is dosed into a living organism by injecting the hollow nano particles of the present invention which are fused with the target-cell substance, the dose can be reduced to 1/100 of that of sole administration of IFN ω or less.

6b. Physiological activity of HBsAg L protein fused with human interferon β1

A nude mouse having PLC/PRF/5 cells derived from human hepatic cancer producing hepatitis B virus surface-antigen protein in the subcutaneous tissue of the back was prepared, and constant production of HBsAg in the blood was confirmed by a HBsAg measurement kit (Dainabot IMx/HBsAg). Next, purified human interferon β1 (IFN β1) expressed by E. coli bacteria was injected subcutaneously into the back of the mouse in an amount of blood concentration of about 200ng/ml using an osmotic pump. Then, the concentration of HBsAg in the blood was measured as needed. After a week, HBsAg was not detected in the blood.

Further, in the case of dosing IFN β1 in an amount of blood concentration of about 10ng/ml, the disappearance of HCV-RNA from the blood after a week was not seen.

In contrast, in the case of dosing purified HBsAg L protein fused with IFN β1 into the mouse in an amount of blood concentration of about 1ng/ml, the disappearance of HBsAg from the blood appeared immediately after a week.

Accordingly, the dose of IFN β1 with respect to HBV was reduced to 1/200 or less at least in a living organism by fusing IFN β1 with HBsAg L protein. More specifically, it was

confirmed that when the target-cell substance IFN β1 is dosed into a living organism by injecting the hollow nano particles of the present invention which are fused with the target-cell substance, the dose can be reduced to 1/200 of that of sole administration of IFN β1 or less.

6c. Physiological activity of HBsAg L protein fused with human HGF

A hepatic tissue (about $10mm^2$) derived from a patient of human hepatic cirrhosis was subcutaneously injected into the renis of a SCID mouse. Next, a commercially-available HGF (human hepatocyte growth factor) was injected subcutaneously into the back of the mouse in an amount of blood concentration of about 10ng/ml using an osmotic pump.

The hepatic tissue of the mouse was observed as needed, and it was confirmed that the image of the implanted hepatic cirrhosis tissue disappeared after two weeks, and a normal hepatic tissue appeared instead.

Further, in the case of dosing HGF in an amount of blood concentration of about 10ng/ml, the disappearance of hepatic cirrhosis tissue after two weeks was not seen.

In contrast, in the case of dosing purified HBsAg L protein fused with HGF into the mouse in an amount of blood concentration of about 1ng/ml, the disappearance of hepatic cirrhosis tissue appeared immediately after a week.

Accordingly, the dose of HGF with respect to the hepatic cirrhosis tissue was reduced to 1/100 or less at least in a living organism by fusing HGF with HBsAg L protein. More specifically, it was confirmed that when the target-cell substance HGF is dosed into the living organism by injecting the hollow nano particles of the present invention which are fused with

the target-cell substance, the dose can be reduced to 1/100 of that of sole administration of HGF or less.

7. I hereby declare that all statements made herein of my own knowledge are true, and that all statements based on experimental data are believed to be true; and further that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: July 20 '07

Shunichi Kuroda